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Detection of Varicella-Zoster Virus DNA in Patients With Acute Peripheral Facial Palsy by the Polymerase Chain Reaction, and its Use for Early Diagnosis of Zoster Sine Herpete

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Varicella-zoster virus (VZV) reactivation without cutaneous vesicles (zoster sine herpete) has been demonstrated in 8 to 25% of patients with acute peripheral facial palsy (APFP) by serological methods. To make an early diagnosis of zoster sine herpete, VZV DNA in oropharyngeal swabs from patients with APFP was examined by the polymerase chain reaction (PCR). VZV DNA was detected in oropharyngeal swabs from 6 of 36 (17%) patients with APFP by PCR. VZV DNA was detected in the oropharyngeal swabs from the six patients at their initial visit (2 to 4 days after the onset of APFP), while the anti-VZV IgM and IgG antibody titers were not increased significantly. In contrast, VZV DNA was undetectable in the oropharyngeal swabs at the time when the VZV specific antibody response appeared. These results indicate that detection of VZV DNA in oropharyngeal swabs by PCR is more useful than currently available serological assays for the early diagnosis of zoster sine herpete in patients with APFP. ***J. Med. Virol. 52:316–319, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: varicella-zoster virus; oropharyngeal swab; Ramsay Hunt syndrome; Bell's palsy

INTRODUCTION

Reactivation of varicella-zoster virus (VZV) is known to cause acute peripheral facial palsy (APFP). Ramsay Hunt syndrome is one of the VZV-associated neurological disease, characterized by zoster around the ears, facial palsy, and eighth cranial nerve symptoms. Furthermore, VZV causes APFP with the absence of skin lesions; such cases are termed zoster sine herpete (ZSH). Using serological methods, significant increases in the anti-VZV antibody titers or the presence of increased anti-VZV IgM antibody titers were observed in 8 to 25% of the patients with APFP [Djupesland et al., 1976; Kukimoto et al., 1988; Tomita et al., 1972; Tomita et al., 1988]. Because VZV-induced facial palsy has a significantly poorer prognosis compared to that of Bell's palsy [Adour, 1994; Robillard et al., 1986], it is important to make an early diagnosis of ZSH and to offer an appropriate therapy.

The early diagnosis of ZSH is often difficult because it takes 2 or 3 weeks to detect a significant increase in serum antibody titers using the complement fixation test. Although the detection of anti-VZV IgM antibody is useful for early diagnosis of VZV infection [Tomita, et al., 1988], the antibody titer often does not increase early in the disease. Furthermore, virus isolation is labor intensive and not very sensitive.

By using the polymerase chain reaction (PCR), VZV DNA has been detected in oropharyngeal swabs and in peripheral blood mononuclear cells (PBMC) from patients with varicella [Ozaki et al., 1990; Ozaki et al., 1994]. In addition, VZV DNA was detected by PCR in cerebrospinal fluid and PBMC from two patients with prolonged radicular pain without zoster rash [Gilden et al., 1994], suggesting that PCR may be useful for the diagnosis of ZSH. In the present study, PCR was used to detect VZV DNA in the oropharyngeal swabs from patients with APFP and we evaluated the usefulness of PCR for the early diagnosis of ZSH.

MATERIALS AND METHODS

Control Viral DNAs

The DNAs of herpes simplex virus (HSV) type 1 strain K192, HSV type 2 strain 20, VZV strain H-N3, VZV strain ONI, and human cytomegalovirus strain KH were obtained from Dr. R. Hondo (Nippon Veteri-

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nary and Animal Science University, Koganei, Japan). DNA extracted from Raji cells (containing Epstein-Barr virus DNA) and the DNA of human herpesvirus-6 OK strain were provided by Dr. H. Kikuta (Hokkaido University, Sapporo, Japan).

Specimens From Patients and Healthy Volunteers

Informed consent was obtained from all patients and healthy volunteers. Thirty-six patients with APFP were examined. The patients visited the hospital within 2 weeks after the onset of the disease and did not have zoster rash in the head and neck region. Eighty-four swabs were collected from the 36 patients with APFP. Four patients with Ramsay Hunt syndrome and two patients with zoster in the dermatome of the fifth cranial nerve were examined as positive controls for VZV reactivation. Oropharyngeal swabs were collected from the six patients and vesicle specimens were taken in five of the six patients. Oropharyngeal swabs were also collected from 15 healthy volunteers, examined, and found to be positive for anti-VZV IgG antibody. Oropharyngeal swabs were suspended in phosphate buffered saline and stored at −80° until use.

Polymerase Chain Reaction

The oropharyngeal swabs were digested with proteinase K (0.1 mg/ml, Boehringer-Mannheim, Mannheim, Germany), and total DNA was prepared by phenol/chloroform extraction and ethanol precipitation. Two pairs of primers that are specific for the major DNA binding protein of VZV [Davison and Scott, 1986] were used to detect VZV DNA using a nested-PCR method. The primers used for the first PCR were VZV 1 (5′-TACGGGTCTTGCCGGAGCTGGTAT-3′) and VZV 2 (5′-AATGCCGTGACCACCAAGTATAAT-3′) as described by Mahalingam et al. [1990]. Two μl of the first PCR product was re-amplified by PCR using VZV 11 (5′-TCTTTCACGGAGGCAAACAC-3′) and VZV 12 (5′-TCCAAGGCGGGTGCATATCT-3′) as the inner primer pair. PCR amplification was performed using a 100 μl reaction mixture consisting of 10 μl of 10X PCR buffer (Perkin Elmer, Norwalk, CT), deoxynucleotide triphosphate mixture (dATP, dGTP, dCTP, and dTTP at a final concentration of 125 μM), 0.5 units of Taq DNA polymerase (Perkin Elmer), and 20 pmol of each primer. Reagents were cycled 30 times on a DNA thermal cycler (TempTronic, Thermolyne, Dubuque, IO), each cycle consisting of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min.

Detection of Amplified Product

One-tenth of the final reaction mixture was electrophoresed on 2% agarose gels containing ethidium bromide (0.7 μg/ml). An amplified band (161-bp) was visible on the gel in samples containing VZV DNA. To confirm that the amplified DNA originated from VZV DNA, the DNA was transferred to nylon membranes (Hybond N, Amersham, Amersham, UK). The amplified DNA was identified using Southern blot hybridization with a digoxigenin-labeled internal oligonucleotide probe (5′-digoxigenin-AATCTCACACGCGCGTGTAACGCGGCTCGA-3′), as described previously [Furuta et al., 1992].

Serological Assays

Sera were taken from all patients with APFP at the initial visit. In 26 patients, paired sera were obtained 2 or 3 weeks thereafter. Anti-VZV IgG and IgM antibody titers were measured using an enzyme-linked immunosorbent assay (Enzygnost Anti-VZV/IgM and IgG, Behringwerke, Marburg, Germany) according to the manufacturer's instructions. Significant changes (over two-fold) in anti-VZV IgG antibody titer or detection of increased anti-VZV IgM antibody titer (greater than 0.1 ELISA value) were considered to indicate VZV reactivation.

RESULTS

Sensitivity and Specificity of the Nested-PCR

The sensitivity of the nested-PCR method was assessed by using a dilution series of the EcoRI-B restriction fragment of VZV strain H-S1 DNA (a gift from Dr. R. Hondo), in the presence of DNA extracted from the oropharyngeal swab of a VZV-seronegative volunteer. An amplified band of 161-bp was detectable on the ethidium bromide-stained agarose gel in samples with concentrations of VZV DNA fragment as low as 0.1 fg (equivalent to approximately one copy of the VZV genome). The DNAs of VZV strains H-N3 and ONI produced positive PCR results using agarose gel analysis and the amplified band was hybridized with the internal oligonucleotide probe by Southern blot hybridization, demonstrating that the 161-bp product was specific to VZV DNA. No amplified fragments were observed on the agarose gel when the DNAs of HSV type 1, HSV type 2, human cytomegalovirus, Epstein-Barr virus, or human herpesvirus-6 were used (data not shown).

VZV DNA in Controls

VZV DNA was detected by PCR in the vesicle fluid and crust from all five patients with zoster and Ramsay Hunt syndrome tested. VZV DNA was detected in oropharyngeal swabs from all of the two patients with zoster in the dermatome of the fifth cranial nerve and from two of the four Ramsay Hunt cases. VZV DNA, however, was not detected in oropharyngeal swabs from the remaining two patients with Ramsay Hunt syndrome. These two patients visited our hospital 8 and 14 days after the onset of zoster, when the skin lesion became crusted. VZV DNA was detected in all oropharyngeal swabs when samples were collected within 3 days after the onset of zoster, while VZV DNA was undetectable at greater than 12 days after onset. VZV DNA was not detected in the oropharyngeal swabs from the 15 healthy VZV-seropositive volunteers (Table I).

TABLE I. Detection of VZV DNA in Oropharyngeal Swabs by PCR

Disease	No. positive cases/ No. tested	No. positive swabs/No. tested Day 0[a]~3	Day 4~7	Day 8~11	Day 12~15	Day 16~
Zoster[b]	2/2 (100%)	1/1	1/1			
Ramsay Hunt syndrome	2/4 (50%)	2/2	0/1	2/3	0/1	0/1
APFP[c]	6/36 (17%)	5/16	1/32	1/14	0/9	0/13
Healthy volunteers	0/15 (0%)					

[a]Day 0 is the day of appearance of zoster rash or facial palsy.
[b]Zoster in the dermatome of the fifth cranial nerve.
[c]Acute peripheral facial palsy.

VZV DNA in Oropharyngeal Swabs From Patients With APFP

VZV DNA was detected in oropharyngeal swabs from 6 of 36 (17%) patients with APFP. In one of the six VZV-positive patients, VZV DNA was detected at the initial visit (day 4) and on day 11 (Fig. 1, case 6). In the other five patients VZV DNA was detected at the initial visit (2 to 3 days after onset), but was not detected thereafter (Fig. 1, case 1 to 5). As with the zoster patients, VZV DNA was usually detected within 11 days after the onset of APFP, but was undetectable beyond 12 days after onset (Table I).

Serological Assays

Anti-VZV IgM antibody was detected in only one patient with APFP at the initial examination (Fig. 1, case 8). The patient visited our hospital 10 days after the onset of the palsy and VZV DNA was not detected in the oropharyngeal swab at that time. Significant changes in anti-VZV IgG antibody titer or detection of increased anti-VZV IgM antibody titer were observed in two patients at the second examination (Fig. 1, case 6 and case 7). In one of the two patients (case 6), VZV DNA was detected in the oropharyngeal swab at the initial visit.

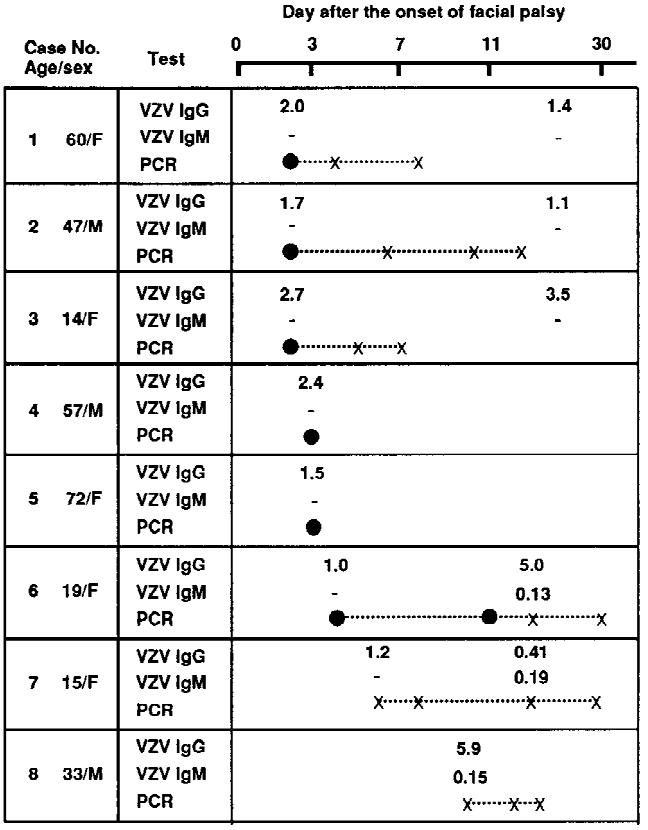

Fig. 1. Eight cases with acute peripheral facial palsy diagnosed as zoster sine herpete by PCR (cases 1 to 5), by serological assay (cases 7 and 8), and by both methods (case 6). Anti-VZV IgG and VZV IgM antibodies were assayed by ELISA. Sera with greater than 0.10 ELISA value were regarded as positive. ● = VZV DNA detected in the oropharyngeal swab by PCR. X = VZV DNA not detected.

DISCUSSION

VZV DNA has been detected frequently in vesicle specimens from patients with zoster using PCR [Dlugosch et al., 1991]. In the present study, VZV DNA was detected in oropharyngeal swabs as well as in vesicle specimens from patients with zoster in the dermatome of the fifth and seventh (around the auricle) cranial nerves when oropharyngeal swabs were collected within several days after the onset of the disease. Latent VZV in the sensory ganglia reactivates and migrates via the nerve to the epithelial surface of the corresponding dermatome. Because the trigeminal and geniculate ganglia of the fifth and seventh cranial nerves are innervated from the oral mucosa, reactivated virus might migrate to the oral epithelium as well as to the skin and replicate there. However, VZV replicates less well in the normal mucosa than in the skin rash. Therefore, VZV DNA was not detected in oropharyngeal swabs beyond 12 days after the onset of zoster, although VZV DNA was always detected in vesicle specimens and crust.

In the present study, VZV DNA was detected in oropharyngeal swabs from six out of 36 (17%) patients with APFP who had no zoster lesions in the oral cavity and skin. The following results clearly suggest an association of VZV detection with the onset of APFP: (1) VZV reactivation causes facial palsy in patients with Ramsay Hunt syndrome and VZV DNA was detected in oropharyngeal swabs from the patients; (2) VZV DNA was not found in oropharyngeal swabs from healthy controls; and (3) VZV DNA in oropharyngeal swabs disappeared by 12 days after the onset of APFP when VZV DNA was detected early in the disease.

It is important to note that VZV DNA could be de-

tected in oropharyngeal swabs at the initial visit (2 to 4 days after onset) when anti-VZV IgM antibody was undetectable. In one of the six VZV DNA-positive patients, anti-VZV IgM antibody and a significant increase in anti-VZV IgG antibody were detected at the second serological assay (paired sera) when VZV DNA in the oropharyngeal swab disappeared. These results indicate that the detection of VZV DNA in oropharyngeal swabs is more useful for early diagnosis of ZSH in patients with APFP than the serological tests. In addition, three VZV DNA-positive patients were found without detectable antibody response to VZV in paired sera. The data suggest that PCR is more sensitive for the detection of VZV reactivation than serological assays. However, VZV DNA was not detected in oropharyngeal swabs at the time when VZV specific antibody response appeared, and VZV DNA in oropharyngeal swabs became undetectable beyond 12 days after the onset of APFP. These findings indicate that PCR has limits, and only oropharyngeal samples collected within 11 days after the onset of APFP might be analyzed by PCR.

In addition to oropharyngeal swabs, PBMC and cerebrospinal fluid have been tested for VZV DNA in patients with VZV infection [Gilden, et al., 1994; Ozaki, et al., 1994]. We were not able to detect VZV DNA in PBMC from 23 patients with APFP, including five cases of ZSH (data not shown). The examination of cerebrospinal fluid requires aseptic procedure and is not carried out routinely in patients with APFP. Oropharyngeal swabs are obtained easily from patients with APFP and the PCR of oropharyngeal swabs appears to be more sensitive than the PCR of PBMC for the detection of VZV reactivation in patients with APFP.

In conclusion, the detection of VZV DNA in oropharyngeal swabs using a nested-PCR method may be a useful test for early diagnosis of ZSH in patients with APFP. Care must be taken, however, regarding the time at which specimens for PCR are collected because VZV DNA in oropharyngeal swabs can be detected only during the acute phase of APFP.

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